INVOLVEMENT OF *PS. FLUORESCENS* PRODUCING SIDEROPHORES IN SUPPRESSION OF *F. OXYSPORUM* F.SP. *GLYCINE* AND STIMULATION OF SOYBEAN NODULATION UNDER IRON – LIMITED CONDITIONS

The severity (% of pre- and post- emergence damping off) of 3 strains of phytopathogenic fungi against soybean plants inoculated with either i.e., *B. japonicum* USDA 110, *Bradyrhizobium japonicum* ARC 501 or *Sinorhizobium* fredii H.H. 303 were recorded under non- limited Fe conditions. The survived plants were harvested after 45 days of planting to record, dry weight of shoots and roots, number and dry weight of nodules and N & Fe uptake in shoots. *F. oxysporum* f.sp. *glycine* and *Bradyrhizobium japonicum* ARC 501 were selected to study their combined interaction with *Ps. fluorescens* strain B in sand culture supplemented 0.2 or 0.5 m mol of Fe as Fe $(OH)_3$ in relation to the above-mentioned parameters. Similar concentrarions of Fe were also applied as Fe EDDHA without *Ps. fluorescens* inoculation to represent control treatments. The different inoculation treatments were re-examined in sandy soil naturally limited in iron with monitoring the same parameters plus total chlorophyll contents of leaves after similar expiremental period. Data showed that the level of disease expression were generally higher in nodulated plants grown in sand culture infested with *F. oxysporum* f.sp. *glycine*. The percentage of survived soybean plants grown in sand culture supplemented with $Fe(OH)_3$ and infested with *F. oxysporium* f.sp. *glycine* was highest when *B. japonicum* ARC 501 was conjugated with *Ps. fluorescens* B. Significant increases were also reported for growth, nodulation and N & Fe uptake due to the above – mentioned dual bacteria inoculation treatment as compared to the single inoculation with *B. japonicum* ARC 501. These findings were confirmed in the records obtained from plants grown in sandy soil naturally limited in Fe content.

Key Words: Soybean, Root nodule bacteria, Phytopathogenic fungi, *Ps. fluorescens*, Fe- supplement, Disease expression, N & Fe uptake.

INTRODUCTION

Although competition between bacteria and fungal plant pathogens for space or nutrients has been known to exist as a biocontrol mechanism for many years, a pronounced interest is presently involved with competition for iron. Therefore, systems such as siderophores, involved in aquisition of iron under iron-limited conditions, may play a role in that competitive interactions **(Kloepper *et al.* 1980)**. Disease suppressions in soils were first attributed to *Pseudomonas* siderophore by **Kloepper *et al.* (1980)**. The role of pyoverdin siderophore produced by many *Pseudomonas* species has been also clearly demonstrated in the control of *Pythium* and *Fusarium* species **(Loper and Buyer 1991).** Seed bacterization with *Pseudomonas* strain RB8 **(Kumar 1998)** or GRP6 **(Rao *et al* . 1999)** reduced the number of chickpea or lentil wilted plants in wilt – sick (*Fusarium oxysporum* f.sp. *ciceri* & *Fusarium oxysporum* f.sp. *lini*) soil, respectively. **Sharma and Johri (2003)** bacterized maize seeds with siderophore – producing *Pseudomonas* strains GRP3A and PRS9 under calcarous soil conditions and in the presence of the phytopathogens,

Sohair A. Nasr ;M. I. Mostafa; Enas A. Hassan and M El-Sawy.
Microbiol. Dept., Fac. Agric., Ain-Shams Univ., Shoubra El-Kheima, Cairo, Egypt.

Colletotrichum dematium, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Corticium rolfsii*. They noticed significant increase in germination percentage and plant growth with 10 μM Fe^{+3} along with bacterial inoculation.

The present study was planned to evaluate the role of siderophores producing *Ps. fluorescens* in suppression of *F. oxysporum* infecting nodulated soybean plants under Fe- limited conditions.

MATERIALS AND METHODS

Soils

Acid-washed sand as well as sandy soil were used for experimental purposes. The sandy soil was collected from Al-Nubaria, Al-Bahaira governorate. The physico-chemical characterstics of that soil are given in **Table (1)**.

Seeds

Soybean (*Glycine max* cv. Crawford) was used as a host plant for experimental studies. Seeds of this host plant were kindly obtained from Field Crops Research Institute, Department of Leguminous Crops, Agricultural Research Centre (ARC), Giza, Egypt

Table (1): Physicochemical characteristics of the soil sample taken from Al-Nubaria to be used in this investigation.

Physical analysis											
Soil particles size distribution (Mechanical analysis)										Soil texture	
$CaCO_3$ %	Gravels > 2 mm %			Sand %			Silt %		Clay %		
7.8	7.4			86.5			8.6		4.9	Sandy soil	
Physical analysis											
pH (1 : 2.5)	SP (%)	ECe (ds / m)	TDS (mg / L)	Water soluble cations and anions (in saturated water extract)							
				Ca (mg / L)	Mg (mg / L)	K (mg / L)	Na (mg / L)	CO_3 (mg / L)	HCO_3 (mg / L)	Cl (mg / L)	SO_4 (ppm)
8.65	24.9	1.65	2976	5.58	2.79	0.19	36.27	0.75	1.5	36.27	6.31
Chemical analysis											
Available nutrients (ppm)										Organic matter (%)	
P	K		Ca	Mg	Fe		Mn		Zn		
Olsen	In Amm .acetata extract				In *DTPA extract						
3.8	12.7		235.0	16.4	1.1		2.3		0.7	0.057	

*DTPA: Ethylene triamine pentaacetic acid

Microorganisms

A range of microbial strains belonging to fluorescent Pseudomonads (*Ps. fluorescens* B) as biocontrol agent, root nodule bacteria (*Bradyrhizobium japonicum* USDA 110, *B. japonicum* ARC 501, *Sinorhizobium fredii* H.H. 303) as symbiotic N_2-fixers and phytopathogenic fungi (*Fusarium solani, F. oxysporum* f.sp. *glycine, Rhizoctonia solani*) were used in this study. Those strains were kindly supplied from different research institutes as shown in **Table (2)**.

Table (2):Microbial isolates or strains used in this study and their sources.

Fluorescent Pseudomonads	Source	Root nodule bacteria	Source	Phytopathogenic fungi	Source
Ps. fluorescens B	Cairo MIRCEN, Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt	*B. japonicum* USDA110 *B. japonicum* ARC 501 *Sinorhizobium fredii* H.H. 303	Culture collection of Biofertilizers Production Unit, Agric. Research Center, Giza, Egypt.	*F. solani* *F. oxysporum* f.sp. *glycine* *Rhizoctonia solani*	Plant Pathology Dept., National Research Center, Dokki, Giza, Egypt Plant Pathology Dept., Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt

Plant nutrient solution

Iron supplements

Fe-EDDHA (ferric ethylenediamine di o-hydroxy phenyl acetic acid) with the trade name sequestrene solurapide Fe 100 SG contained 6% Fe, and $Fe(OH)_3$ (ferric hydroxide) contained 52.14% Fe were used as sources of iron in pot experiments.

Mineral nutrients

The N free nutrient solution of **Somasegran and Hoben (1985)** was used to feed the leguminous host plants grown in sand culture and potassium nitrate (13.86 % N) was used as a mineral fertilizer .

Experimental techniques

Performance of nodulated soybean plants in soil infested with phytopathogenic fungi

The interactions among 3 active strains of root nodule bacteria and 3 strains of phytopathogenic fungi was studied on soybean grown in sterilized sand culture.

Seed inoculation and sand infestation

Soybean seeds selected for uniformity in size and viability (95%) were surface sterilized with ethanol (95%) and 0.1% $HgCl_2$ for 30 min, followed by several washing changes by sterilized distilled water. Seeds were then sown in pots containing 2 Kg acid washed sand amended with 20 mg N/kg as potassium nitrate. Ten ml liquid cultures (containing about 10^8 CFU / ml) of each of the above-

mentioned 3 strains of root nodule bacteria were pipetted on soybean seeds at the time of planting. Sand infestation with either of the 3 above-mentioned phytopathogenic fungi was carried out by applying 10 ml / pot of *F. solani*, or *F. oxysporum* f. sp. *glycine* spores suspension, (containing about 10^5 spore / ml) *Rh. solani* was applied as air dried and ground inoculum at a rate of 1%.

Treatments

Fifteen treatments were adopted comprising

A- Seed inoculation with

a- *Bradyrhizobium japonicum* USDA 110

b- *Bradyrhizobium japonicum* ARC 501

or c- *Sinorhizobium fredii* H.H 303

B- Sand infestation with

a- *Fusarium oxysporum* .f.sp. *glycine*

b- *Fusarium solani*

or c- *Rhizoctonia solani*

C- The dual application of the above – mentioned treatments, i.e., sand infestation with phytopathogenic fungi and seed inoculation with root nodule bacteria

Five replicates were made for each treatment. Plants were left to grow under net – house conditions and fed twice weekly with N free nutrient solution **(Somasegran and Hoben 1985)**.

Parameters measured

Plants were harvested after 45 days from sowing to record:

a- level of disease expression: the percentage of pre- and post- emergence damping off along with that of survived plants according to the following equations:

% Pre – emergence damping off =

$$\frac{\text{No. of non-emerged seeds}}{\text{No. of sown seeds}} \times 100$$

% Post – emergence damping off =

$$\frac{\text{No. of dead seedlings}}{\text{Total No. of emerged seedlings}} \times 100$$

b- Number (/plant) and dry weight of nodules(mg/plant).

c- Dry weight of shoots and roots (g/plant) by oven drying at 70 C° up to constant weights.

d- Nitrogen and iron uptake according to **Jackson (1967).**

Evaluation of the role of *Pseudomonas fluorescens* as a biological control agent against *F. oxysporum* f.sp. *glycine* infecting soybean

Acid washed sand amended with 20 mg N/kg as KNO_3 and 0.2 or 0.5 m mol Fe as $Fe(OH)_3$ was used to evaluate the role of *Pseudomonas fluorescens* B as biological control agent against *Fusarium oxysporum* f.sp. *glycine* infecting soybean. Similar concentrations of Fe were also applied as Fe- EDDHA without *Pseudomonas fluorescens* inoculation to represent control treatments. Sandy soil naturally limited in Fe content **(see Table 1)** was packed in pots with 2 Kg capacity and then infested with *Fusarium oxysporum* f.sp. *glycine*. Surface sterilized soybean seeds were inoculated with *Bradyrhizobium japonicum* ARC 501 singly or conjugated with *Ps. fluorescence* B. Sand infestation and seed inoculation were carried as mentioned above. In dual bacterial inoculation treatment, *Pseudomonas fluorescens* was pipetted at a rate of 10 ml (containing 10^8 CFU/ ml) on soybean seeds at the time of planting. Each treatment was represented by four replicates. Pots were kept under net-house conditions and fed with **Somasegaran and Hoben (1985)** nutrient solution (minus iron). Percentage of pre and post emergence damping-off were record after 1 and 3 weeks from sowing, respectively. After 45 days from planting, survived plants were harvested to record the number and dry weight of nodules, dry weight of shoots and roots, nitrogen and Fe uptake. Total chlorophyll content was only determined in leaves samples of plants grown in sandy soil according to the method described by **Arnon (1949).**

RESULTS

Performance of nodulated soybean in sand infested with phytopathogenic fungi under un-limited iron condition

Disease expression

Inoculation of soybean with either of the 3 root nodulators seemed to interact with the severity of pre or post emergence damping off induced by the tested phytopathogenic fungi **(Table 3)**. The levels of the 2 above-mentioned disease expression were generally lower in plants grown in sand infested with *F.solani* plus root nodulating bacteria than in control treatment (fungal inoculation alone). This effect resulted in the highest percentages of plant survival being 49.00, 51.89, 46.12 and 42.67% in *B. japonicum* USDA 110, *B. japonicum* ARC 501, *S. fredii* H.H. 303 and control treatment, respectively. On the other hand, the levels of disease expression appeared to increase in nodulated plants grown in sand infested with *Rh. solani* compared to fungal inoculation alone. The pre-and post- emergence damping off records of nodulated plants grown in sand infested with *F. oxysporum* f.sp. *glycine* gave checkered view. However, the lowest percentages of survived plants were generally recorded in latter fungal treatments.

Growth, nodulation and nutrient uptake

Plants grown in infested sand and un-inoculated with root nodule bacteria gave lower records of total dry weight and N as well as Fe uptake **(Table 4)** than those inoculated with either of the bacterial nodulators. Interacting treatments showed that sand fungal infestation in the presence of either of the 3 symbiotic N_2 fixers also reduced the above – mentioned growth parameters (plus nodulation) but to an intermediate rank between the records of fungal and bacterial controls. However, *B. japonicum* ARC 501 was generally superior to other endophytes in the records of nodule number, nodule dry weight, N and Fe uptake in dual inoculation treatments.

Table (3): Pathogenicity of 3 strains of phytopathogenic fungi against soybean *(Glycine max* cv. Crawford *)* inoculated with either of 3 strains of symbiotic nitrogen fixers under un-limited iron condition.

Inoculatoin treatment	Disease Expression											
	Control			*Fusarium solani*			*Fusarium oxysporum* f. sp. *glycine*			*Rhizoctonia solani*		
	% pre - emergence	% post - emergence	% survival	% pre – emergence damping off	% post - emergence damping off	% survival	% pre – emergence damping off	% post - emergence damping off	% survival	% pre – emergence damping off	% post - emergence damping off	% survival
Uninoculated (control)				35.22 c	22.11 de	42.67 e	42.66 a	25.00 e	32.34 h	30.04 e	22.30 de	47.66 be
Bradyrhizobium japonicum USDA110	0.0	0.0	100	30.00 de	21.00 e	49.0 b	43.10 a	21.49 e	35.41 g	32.10 d	25.00 b	42.90 e
Brady. japonicum ARC 501	0.0	0.0	100	29.11 e	19.00 f	51.89 a	38.00 b	24.00 bc	38.00 f	32.00 d	24.00 bc	44.00 de
*Sinorhizobium fredi*i H.H303	0.0	0.0	100	32.09 d	20.99 e	46.12 c	38.21 b	26.77 a	35.02 g	31.98 d	23.00 cd	45.02 d

- Plants were fed with nutrient solution contained $FeCl_3$
- Means in a column not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

Table (5): Interactions between phytopathogenic fungi and root nodule bacteria in relation to growth,nodulation and N &Fe uptake of soybean (*Glycine max* cv. crawford) under un-limited iron conditions.

Treatment		Total dry weight (g / plant)	No. of nodules (/ plant)	Dry wt. of nodules (mg/plant)	Nitrogen Uptake (mg/shoot)	Fe- Uptake (ppm/shoot)
Fusarium solani		1.73 def	-	-	10.51 i	128 f
Fusarium oxysporum f.sp. glycine		1.68 def	-	-	11.55 i	130 f
Rhizoctonia solani		1.67 ef	-	-	11.11 i	120 g
Brady. Japonicum USDA 110		2.22 b	14 d	90 ef	24.15 c	169 b
Brady. japonicum ARC 501		2.63 a	19 a	120 a	40.60 a	190 a
Sinorhizobium fredii H. H303		2.20 b	15 cd	95 cd	25.76 b	170 b
Fusarium solani	*Brady. japonicum* USDA 110	1.82 cdef	9 f	70 h	13.42 h	146 de
	Brady. japonicum ARC 501	1.89 cde	15 cd	95 cd	15.24 fg	158 c
	Sinorhizobium fredii H.H303	1.81 cdef	11 e	86 f	13.31 h	146 de
Fusarium oxysporum f. sp *glycine*	*Brady. japonicum* USDA*110*	1.79 cdef	8 f	76 g	14.16 gh	144 e
	Brady. japonicum ARC 501	1.95 cd	16 bc	100 b	19.28 a	153 cd
	Sinorhizobium fredii H.H303	1.89 cde	12 e	90 de	15.74 fg	149 de
Rhizoctonia solani	*Brady. japonicum* USDA 110	1.69 def	12 e	79 g	13.00 h	145 de
	Brady. japonicum ARC 501	2.00 bc	17 b	99 bc	22.65 d	160 c
	Sinorhizobium fredii H.H303	1.91 f	12 e	90 ef	16.63 f	148 de

- Plants were fed with nutrient solution contained $FeCl_3$

- Means in a column not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

Role of *Ps. fluorescens* B as a biological control agent against *F. oxysporum* f.sp. *glycine* under limited iron conditions

In sand culture

Data in Table (5) present the percentages of pre- and post-emergence damping off and survived soybean plants when inoculated with *B. japonicum* ARC 501, and grown in sand infested with *F. oxysporum* f.sp. *glycine* and amended either of 2 forms of iron varied in availability, i.e., Fe-EDDHA and $Fe(OH)_3$. With the 2 concentrations (0.2 and 0.5 m mol Fe) of the scarcely available $Fe(OH)_3$, *Ps. fluorescens* B was coinoculated with *B. japonicum* ARC 501 onto soybean seeds. It is obvious that in sand supplemented with Fe-EDDHA, higher percentages of pre-emergence damping off were recorded being 55 and 60% in 0.2 and 0.5 concentrations, respectively. The percentages were quite reduced when *Ps. fluorescens* B was coinoculated with *B. japonicum* ARC 501 in sand supplemented with either of the 2 $Fe(OH)_3$ concentrations. The latter treatments also gave considerably lower records of post-emergence damping off. The reduction in those two symptoms of disease expression was reflected in increased percentages of soybean survived plants. This finding could be examplified by the 2 fold increase in the % of survived plants grown with *Ps. fluorescens* B and supplemented with 0.2 m mol Fe as $Fe(OH)_3$ over those supplemented with the same concentration of Fe - EDDHA (control treatment). Plants grown under the former treatment also showed better growth performance, nodulation, N and Fe uptake (Table 6) than the latter. This observation is represented by records of 2.50, 21, 120, 37 and 185 against 1.90, 11, 70, 28.9 and 150 for total dry weight (mg/ plant), nodule number (/plant), nodules dry weight (mg/ plant), N uptake (mg/ shoot) and Fe uptake (ppm/ shoot), for plants grown with 0.2 m mole Fe as Fe $(OH)_3$ or Fe – EDDHA, respectively.

Table (5): Effect of siderophores producing *Pseudomonas fluorescens* B on the pathogenicity of *Fusarium oxysporum* f.sp. *glycine* against soybean inoculated with *Bradyrhizobium japonicum* ARC 501 grown in the presence of 2 concentrations of iron as $Fe(OH)_3$.

Inoculation treatment	Iron form	m mol Fe	Disease Expression		
			% Pre-emergence damping off	% Post-emergence damping off	% survival
Fusarium oxysporum f.sp. *glycine* + *Bradyrhizobium japonicum* ARC 501	Fe-EDDHA (control)	0.2	55 b	15 c	30 e
		0.5	60 a	20 b	20 f
	$Fe(OH)_3$	0.2	33 c	25 a	42 c
	$Fe(OH)_3$ + *Ps. fluorescens* B		22 d	14 c	64 a
	$Fe(OH)_3$	0.5	35 c	26 a	39 d
	$Fe(OH)_3$ + *Ps. fluorescens* B		23 d	16 c	63 a

Means in a column not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

Table (6): Effect of siderophore producing *Pseudomonas fluorescens* B on growth, nodulation and N & Fe uptake of soybean *(Glycine max* cv. Crawford*)* inoculated with *Bradyrhizobium japonicum* ARC 501 and grown in sand infested with *Fusarium oxysporum* f. sp. *glycine* in the presence of 2 concentrations of iron as $Fe(OH)_3$.

Inoculation treatment	Iron form	m mol Fe	Total dry weight (g / plant)	No. of nodule (/ plant)	Dry wt. of nodules (mg/plant)	Nitrogen uptake (mg/shoot)	Fe Uptake (ppm / shoot)
Fusarium oxysporum f.sp. *glycine* + *Bradyrhizobium japonicum* ARC 501	Fe-EDDHA (control)	0.2	1.90 c	11de	70 e	28.90 c	150 d
		0.5	1.97 c	10 e	66 f	31.15 b	160 c
	$Fe(OH)_3$	0.2	1.57 d	15 c	101 c	19.03 e	135 e
	$Fe(OH)_3$ + *Ps. fluorescense* B		2.50 a	21 a	120 a	37.00 a	185 b
	$Fe(OH)_3$	0.5	1.64 d	13 cd	99 d	21.21 d	136 e
	$Fe(OH)_3$ + *Ps. fluorescense* B		2.45 a	20 a	115 b	36.67 a	190 a

Means in a column not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

In sandy soil

The role of *Ps. fluorescens* B as a biocontrol agent against *F.oxysporum* f.sp. *glycine* infecting soybean was retested in sandy soil naturally limited in Fe content. It is clear that soybean plants inoculated with *B. japonicum* ARC 501 and grown in soil infested with *F.oxysporum* f.sp. *glycine* showed higher percentages of disease expression than when conjugated with *Ps. fluorescens* B. The recorded figures were 31.5, 20.1 against 12.3 and 9.8 %, in the above-mentioned respective order **(Table 7)**. Thus, the percentages of survived plants were 48.4 and 77.8 % in dual and triple microbial treatment, respectively. No significant differences were observed in chlorophyll content, total dry weight, nodulation and N as well as Fe uptake of nodulated soybean plants escaped from post damping off and those only inoculated with *B. japonicum* ARC 501 **(Table 8)** . However, plant inoculated with *B. japonicum* ARC 501 + *Ps. fluorescens* B and survived in the infested soil showed significantly higher levels of performance in all tested parameters **(Table 8)** compared with other treatments. This observation was particularly obvious in total chlorophyll content, total plant dry weight, nodule number and nodule dry weight, N and Fe uptake where about 86, 57, 45, 14, 121 and 29% increases were recorded in triple compared with dual inoculation treatment, respectively.

Table (7) :Effect of siderophores producing *Ps. fluorescens* B on :pathogenicity of *Fusarium oxysporum* f.sp. *glycine* against soybean inoculated with *Bradyrhizobium japonicum* ARC 501 and grown in Fe limited sandy soil.

Inoculation treatment	Disease Expression		
	% Pre-emergence damping off	% post - emergence damping off	% Survival
B. japonicum ARC 501	0 .0 c	0.0 c	100.0 a
B. japonicum ARC 501 + *F. oxysporum* f.sp. *glycine*	31.5 a	20.1 a	48.4 c
B. japonicum ARC 501 + *F. oxysporum* f.sp. *glycine* + *Ps. fluorescens* B	12.3 b	9.8 b	77.8 b

Means in a column not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

Table (8): Effect of siderophore producing *Ps. fluorescens* strains B on growth, nodulation and N & Fe uptake of soybean *(Glycine max* cv. crawford*)* inoculated with *Bradyrhizobium japonicum* ARC 501 and grown in Fe limited sandy soil infested with *Fusarium oxysporum* f. sp. *glycine.*

Parameter	Treatment		
	***B. japonicum* ARC 501**	***B. japonicum* + *F. oxysporum* f.sp. *glycine***	***B.japonicum* + *F. oxysporum* f.sp. *glycine* + *Ps. fluorescens* B**
Total chlorophyll content (mg / g leaves)	7.40 b	7.60 b	14.10 a
Total dry weight (g / plant)	2.18 b	2.04 b	3.21 a
No. of nodules (/ plant)	24.00 b	22 b	32.00 a
Nodules dry weight (mg / plant)	109.00 b	105 b	120.00 a
Nitrogen uptake (mg/shoot)	30.56 b	28.16 b	62.29 a
Fe uptake (ppm/shoot)	161.00 b	157.00 b	202.00 a

Means in a raw not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

DISCUSSION

Biological control (biocontrol) of soil borne plant pathogens by indigenous microbes, or by introduction of antagonistic microorganisms to the soil, is a potential non chemical mean for plant disease control. There is a substanial evidence to believe that siderophores play some role in the biocontrol of phytopathogenic microorganisms by sequestering iron and thereby inhibiting their growth or metabolic activity **(Misaghi *et al.* 1982).** Disease suppressions in soils were first attributed to *Pseudomonas* siderophore by **Kloepper *et al.* (1980)**. Although many rhizosphere *Pseudomonas* species are plant-pathogenic, some have been shown to promote plant growth and inhibit pathogenic bacteria and fungi by producing siderophores.

In this study, the fusarial wilt suppression and soybean response improvements were observed in treatment comprising *B. japonicum* ARC 501 plus the siderophores producing *Ps. fluorescens* B co-inoculation. This finding was reported in acid washed sand supplemented with Fe $(OH)_3$ and also in sandy soil naturally limited in Fe content. The elevated expression of the disease under conditions of available Fe as Fe- EDDHA may indicate the strong involvement of siderophores in disease suppression. **Becker and Cook (1988)** in his study on *Pseudomonas* strain B324

strongly supported previous reports of **Loper (1988)** and **Bakker *et al.* (1986)** that siderophore production is an important mechanism by which some strains of bacteria protect plant against root pathogens. This conclusion was based on several lines of evidence, including : (1) strain B 324 was inhibitory to all seven *Pythium* isolates on KMB agar (a medium with low iron content) but was not inhibitory to any of the isolates on PDA (a medium with high iron content), suggesting that the inhibitory effect of this strain results from production of siderophore and not of antibiotics; (2) both the inhibitory effect of the B 324 strain on KMB agar and its growth – promoting activity on wheat in the natural Thatuna silt loam soil were reduced or nullified by the addition of $FeCl_3$ to the KMB agar or to the soil; (3) the synthetic iron chelator EDDHA added to the soil resulted in a growth promoting effect on wheat equal to that obtained with strain B 324; and (4) siderophore – negative mutants of B324 R were less effective than the parent strain or ineffective in growth promotion of wheat.

Hoseong *et al.* (1999) reported that siderophore – producing *Pseudomonas fluorescens* GL20 considerably inhibited spore germination and hyphal growth of *Fusarium solani* in dual culture. The study of the antifungal mechanism with *Ps. fluorescens* GL20 – S101 (sid), a mutant defective in siderophore synthesis, confirmed that the production of siderophores was responsible for the fungal inhibition by siderophore – mediated Fe (III) competition under iron – deficient conditions. In pot trials of bean (*Phaseolus vulgaris*) inoculated with *Ps. fluorescens* GL20, disease incidence was remarkably reduced up to 94% compared with *F. solani* alone and plant growth was also increased nearly 1.6 fold compared with that of the untreated control (**Hoseong *et al.* 1999**). Randomized block design (RBD) analysis of sickpot studies (soil inoculated with phytopathogens) with an MACS 124 variety of soybean, bacterized with siderophoregenic *B. japonicum*, showed a marked increase in the percentage of germination, nodulation, chlorophyll, oil and protein content as well as number of pods. Field trial study confirmed the pot results, which were evident from enhancement in shoot length, number of branches, chlorophyll content, number of nodules, root length and number of pods **(Khandelwal *et al.* 2002)**. The deleterious effect of *R. solani* was also decreased on soybean when co-inoculated either with *B. japonicum* SB-12 or *Pseudomonas* JLOZ-3, registering an increase in germination, seedling stand, dry matter accumulation and nutrient uptake over the treatment with *R. solani* alone **(Zaidi, 2003)**. Triple inoculation with SB – 2 + JIOZ – 3 + *R. solani* completely eliminated the ill effects of *R. solani* with recording a higher seed emergence, growth, nodulation and nutrient uptake than the uninoculated control.

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ارتباط السيدوموناس فلوروسينس المنتجة للسيدروفورس بتثبيط فطر *Fusarium oxysporum* f.sp. *glycine* و تحسين تعقيد فول الصويا تحت ظروف نقص الحديد

سهير أحمد ابراهيم نصر – مجدى اسماعيل مصطفى –
ايناس عبد التواب حسن – محمد الصاوى مبارك
قسم الميكروبيولوجيا، كلية الزراعة، جامعة عين شمس، شبرا الخيمة ، القاهرة، مصر

تم فى هذه الدراسة تسجيل نسب الإصابة بالذبول المبكر والمتأخر فى نبات فول الصويا الملقحة لسلالات البكتريا العقدية *Sinorhizobium fredii* H-H303 , *Bradyrhizobium japonicum* ARC 501 ، *Bradyrhizobium japonicum* USDA 110 والنامية تحت ظروف توافر الحديد بالمزارع الرملية المحقونة بواحد من ثلاثة سلالات فطرية ممرضة للنبات ، وقد حصدت النباتات التى لم تصاب بالذبول بعد ٤٥ يوم من الزراعة، لتقدير الوزن الجاف للمجموع الخضرى والجذرى، الأعداد والأوزان الجافة للعقد الجذرية وكمية النيتروجين والحديد الممتص بالمجموع الخضرى، ثم انتخبت السلالتين *Fusarium oxysporum* f.sp. *glycine*, *Bradyrhizobium japonicum* ARC 501 لدراسة تأثيرهما المشترك مع السلالة *Pseudomonas fluorescens* B المنتجة للسيدروفورس ، على المقاييس المشار اليها تحت ظروف المزرعة الرملية المضاف اليها ٠,٢ ، ٠,٥ ملليمول من الحديد على صورة هيدروكسيد حديديك ، وأضيفت نفس التركيزات من الحديد على صورة Fe-EDDHA دون تلقيح بسلالة السيدوموناس لتمثل معاملتى مقارنة، ثم أعيد اختبار تأثير معاملات التلقيح السابق على المقاييس المشار اليها فى تربة رملية تتسم بقلة محتواها من الحديد بصورة طبيعية ولنفس الفترة التجريبية. وقد أظهرت النتائج أن مستويات الإصابة كانت أعلى فى النباتات الملقحة *Bradyrhizobium* والنامية فى المزارع الرملية المحقونة بفطر *Fusarium oxysporum* f.sp. *glycine* ، وسجلت أعلى نسبة من النباتات الغير مصابة فى معاملة التلقيح المزدوج بـ *Bradyrizobium japonicum* ARC 501 ، *Pseudomonas fluorescens* B ، مقارنة بالـ *Bradyrhizobium* فقط فى المزارع الرملية المسمدة بهيدروكسيد الحديديك وارتبط ذلك بزيادة معنوية فى النمو والتعقيد وامتصاص النيتروجين والحديد ، وقد تأكدت هذه النتائج فى القياسات المسجلة للنباتات النامية فى التربة الرملية قليلة المحتوى من الحديد طبيعياً.